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Sources of Energy for Increased Metabolic Demand During Metamorphosis of the Abalone *Haliotis rufescens* (Mollusca)

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Abstract. Pelagic, lecithotrophic (nonfeeding) larvae of the red abalone (*Haliotis rufescens*) settle and subsequently metamorphose into benthic juveniles capable of feeding on particulate food. Thus, metamorphosis must be fueled by either endogenous reserves or a nonparticulate food source such as dissolved organic material (DOM) in seawater. The metabolic rates (measured as oxygen consumption) of abalone larvae were found to increase by an average of 3- to 5-fold from the larva to early juvenile stage. The total cost of development from embryo to juvenile measured for three cultures ranged from 41.6 mJ to 55.0 mJ. Meeting this cost would require 1.3 to 1.7 μg of biomass (ash-free dry mass), which is similar to the initial biomass of the spawned oocyte at 1.36 ± 0.04 μg (mean of four cultures). However, there was no net loss of biomass during development from the oocyte to the juvenile. The uptake of alanine and glucose from seawater by larvae and juveniles could provide one-third of the organic material required to supply metabolism, even if the transporters were only operating at 20% of their maximum capacity throughout development. For larvae undergoing metamorphosis (between 6- and 9-days-old) the proportion of total metabolic demand supplied using aerobically catabolized biomass was only 39%. The higher metabolic rates of metamorphosis are met only in part by consuming stored endogenous reserves. Concomitant with an increase in mass-specific metabolic rate during metamorphosis, the maximal capacity (J_{max}) for the transport of dissolved alanine from seawater increased 3-fold, from 61.2 ± 1.9 (SE) to 182.0 ± 49 pmol alanine individual^{-1} h^{-1}. The majority (range: 61% to 100%) of the energy requirements of larval and early juvenile development of *H. rufescens* could be supplied by input of DOM from the environment. Measurements of transport rates of amino acids and sugars by these animals, and calculations of the energy input from these substrates, indicate that the cumulative transport of DOM from seawater during development to the early juvenile stage could supply an amount of energy equivalent to the initial maternal endowment of energy reserves to the oocyte of this lecithotrophic species.

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Introduction

Metamorphosis of marine invertebrates from a pelagic larva to a juvenile can involve reorganization of existing tissue and construction of new tissue (*e.g.*, ascidians: Cloney, 1961; echinoderms: Hinegardner, 1969). For many species, the processes involved in metamorphosis take place after the larva has settled to the benthos in response to environmental cues (Hadfield, 1986). For example, haliotid larvae settle on encrusting red algae (Morse and Morse, 1984) and subsequently metamorphose over the next 2 to 7 days into juveniles (Crofts, 1937). During this time, they lose their velum, develop enlarged gills and foot, and begin deposition of the adult shell. The rearrangement of tissues at metamorphosis is potentially energetically costly and may result in depletion of endogenous reserves. Lucas *et al.* (1979) reported that metamorphosing barnacle cyprids (*Balanus bala-*

noides) showed increases in metabolic rates while energy reserves (protein and lipid) decreased. Similarly, newly settled oyster larvae (*Ostrea edulis*) used endogenous lipid reserves (Holland and Spencer, 1973), or lipid and protein (Rodriguez *et al.*, 1990), in the transition from larva to settled spat. It is still unclear, however, to what extent larval energy reserves affect post-metamorphic success (Highsmith and Emlet, 1986; Pechenik and Eyster, 1989).

The rate of depletion of energy reserves for embryos and larvae of several species of marine invertebrates is insufficient to meet total metabolic demands during development; although sufficient energy is available in the egg, these reserves are not used (Jaeckle and Manahan, 1989a; Shilling and Manahan, 1990). The conclusion from these studies was that uptake of DOM from seawater was providing the missing energy. The reports to date of the physiology of metamorphosis of marine invertebrates have focused mainly on the role of endogenous reserves (*e.g.*, Holland and Spencer, 1973; Rodriguez *et al.*, 1990). However, bivalves undergoing metamorphosis can absorb dissolved amino acids from seawater during metamorphosis (Manahan and Crisp, 1983), although the quantitative importance of this process to metabolism is unknown. Haliotid larvae cannot feed on particles (Crofts, 1937) but do have the capacity to transport dissolved organic material (DOM, *e.g.*, amino acids) from seawater (Jaeckle and Manahan, 1989b) and do not use endogenous reserves to meet the total requirements of metabolism (Jaeckle and Manahan, 1989a). These observations lead to the suggestion that DOM could be important to larval energetics. In the present study, we address three questions: (1) What are the metabolic costs during complete metamorphosis of abalone larvae (*H. rufescens*)? (2) What proportion of these costs can be met through utilization of endogenous reserves? (3) How much energy does exogenous DOM contribute to the energetics of metamorphosis?

Materials and Methods

Culturing of larvae and plantigrades

Batches of fertilized abalone oocytes, from seven separate spawnings, were cultured from the zygote to up to 6 days after settlement (juvenile) in flowing 5-μm-filtered, UV-irradiated seawater at a commercial abalone hatchery (the AbLab, Port Hueneme, CA). The temperatures at which the larvae were reared, and the corresponding temperatures used for physiological measurements, varied with time of year. The following is a list of the cultures by letter designation, with month and temperature ($\pm$1°C): Culture A—March, 12°C; B—May, 13°C; C—June, 15°C; D—July, 20°C; E—February, 13°C; F—March, 12°C; G—May, 15°C. Measurements of ash-free dry weight (biomass) and all physiological measurements were not done on all stages or for all seven cultures (specifics given in Results). All stages were maintained on 80-μm-mesh screens that were immersed in the running seawater. Veliger-stage larvae were induced to settle with γ-amino butyric acid (GABA, Morse *et al.*, 1979) once they had attained metamorphic competency (5 to 9 days post-fertilization). Under the conditions in use in the commercial abalone hatchery at the time of our experiments, a concentration of 100 μM GABA with a 30-min exposure was used to induce metamorphosis.

The stages of larvae and plantigrades (early post-settlement) used were characterized according to various morphological attributes (*e.g.*, presence of velum). Live animals were observed under dissecting and compound microscopes. The stages were defined as follows: 0—unfertilized oocyte; i—veliger not competent to settle; ii—swimming veliger competent to settle, has velum, branched cephalic tentacles, eyes, and the capacity to crawl briefly on its foot; iii—newly settled larva no longer swimming (referred to as a plantigrade), but with similar morphology to stage ii; iv—metamorphosing plantigrade, which has lost velum, adult shell is growing from edge of larval shell, gill buds are apparent, mouth parts are developing, and foot is larger. Stage ii is equivalent to stage 21 as defined by Hahn (1989) for *H. rufescens*, and stage iv is equivalent to stage 4 (for plantigrades) for *H. discus hannai* (Hahn, 1989).

The possibility that plantigrades (stage iii) and juveniles (stage iv) were feeding on particulate material was assayed by placing the animals on a glass slide coated with diatoms and bacteria and observing the animals with a compound microscope. When present, the mouth and radula were readily visible, as were the feeding tracks left by the feeding juvenile. All settled stages were tested in this way. The slides were coated by placing them either in ambient, continuously running, unfiltered seawater for several days or in the presence of cultured unialgae (*Thalassiosira pseudonana, Dunaliella tertiolecta,* and *Rhodomonas* sp.).

Measurement of metabolic rates

Oxygen consumption was measured for swimming larvae and settled plantigrades using coulometric respirometry. This technique allows long-term, continuous measurement of oxygen consumption rates by replacing the oxygen as it is depleted from the respiration chamber. The apparatus and methods used were those of Heusner *et al.* (1982) that have been adapted for use with marine invertebrate larvae (Hoegh-Guldberg and Manahan, 1995). Glass respirometry chambers were sterilized with 70% ethanol and rinsed thoroughly with sterile-autoclaved seawater. Swimming larvae or crawling planti-

grades were rinsed with autoclaved seawater and placed into the respirometry chambers in 1 to 2 ml of filtered (0.2-μm pore-size), autoclaved seawater (the volume of seawater does not affect the measurement). The number of individuals per chamber ranged from 22 to 911 (larvae) and 44 to 243 (plantigrades). The measurement of absolute rates of oxygen consumption by larvae of marine invertebrates is not affected by larval densities in the ranges used in the present study (Hoegh-Guldberg and Manahan, 1995). After equilibration to temperature and pressure (1 to 4 h), recording commenced and was continued for 6 to 30 h. The seawater within the chambers was not stirred. The basis of the coulometric respirometry technique does not require stirring for accurate measurement of oxygen consumption (Heusner *et al.*, 1982). Each set of measurements was conducted at the temperatures at which the larvae and plantigrades were cultured. One respiration chamber containing seawater, but without animals, was run in parallel as a control for oxygen depletion that was not due to the presence of the animals. Jaeckle and Manahan (1989b) found very few bacteria attached to abalone larvae (<6 per larva) using epifluorescence and scanning electron microscopy, thus the contribution of bacterial metabolism to measured respiration rates is probably below the limit of detection. About every 24 h the animals in the chambers were replaced with new animals from the same culture, at which time the chambers were cleaned again. New animals were used to reduce differences in metabolism that might exist between the cultured animals (used for biomass and nutrient transport determinations) and those maintained in the respirometer. At the end of each time period the animals in each chamber were removed and counted to allow for the calculation of metabolic rates on a per-animal basis.

A second technique for measuring rates of oxygen consumption was also used, the Winkler titration method (Parsons *et al.*, 1984). This involved measuring the depletion of oxygen in a sealed BOD (biological oxygen demand) bottle (temperature = 15°C) that contained swimming veligers (stage ii, Culture G, 7-day-old). Six BOD bottles (volume = 300 ml each) were filled with larvae suspended in filtered seawater (0.2 μm pore-size) with densities ranging from 651 to 1987 larvae per bottle. One bottle containing only filtered seawater (control) was incubated for 6 h, as were the bottles containing larvae. The seawater within the bottles was not stirred during incubation. All of the oxygen in the bottles was then chemically fixed and the Winkler titration conducted. The larvae in each bottle were removed and counted immediately after the chemical fixing and measurement of oxygen was complete. The larval shells and attached tissue were easily visible after the chemical fixation for Winkler titration.

Biomass determination (ash-free dry weight)

Net changes in total biomass per individual were measured to determine how much of the metabolic cost was met by depletion of endogenous reserves. Six replicate samples per day were taken for each culture for biomass determination. Biomass of the various developmental stages was determined as described by Jaeckle and Manahan (1989a). Briefly, the organisms were washed with filtered (0.2 μm pore-size) 3.4% ammonium formate (w/v), to replace sea-salts, placed in an aluminum dish, and dried at 80°C. The dried tissue was weighed, ashed at 450°C for 5 to 6 h, and weighed again. For a given culture, all sampled stages of development were stored frozen (−20°C) until they were dried, ashed, and weighed together. This was done to reduce any effects from the drying and ashing treatments on comparisons among stages for a given culture. The difference between the ash weight and dry weight is the ash-free dry organic weight, here defined as biomass. The changes in biomass were converted into energy by using the enthalpic equivalents given by Gnaiger (1983). Comparisons between mean biomasses were conducted using analysis of variance (ANOVA; Zar, 1984). When a difference was observed using ANOVA within a culture, a multiple comparison test (Newman-Keuls) was conducted to permit comparisons among biomasses for different days. Errors are presented either as standard error of the mean (SEM) or 95% confidence intervals (95% CI).

Measurement of amino acid and sugar transport rates

The kinetic constants for glucose and alanine transport were measured for swimming larvae and crawling plantigrades to determine what proportion of the metabolic costs during metamorphosis could potentially be supplied by transport of exogenous dissolved amino acids and sugars. The substrate concentrations used for these kinetic experiments ranged from 0.1 to 100 μM, with 50 to 300 animals being exposed to radioactively labeled substrates in 2 ml of sterile-filtered seawater. Measurements were conducted at the temperatures at which the organisms were cultured. A technique for measuring rates of transport was employed which was very similar to that used by Manahan (1983) for other molluscan larvae, where animals were exposed undisturbed for a short time to a known concentration of substrate added to seawater. This technique was used in the present study because the periodic disturbances of late-stage abalone larvae associated with other transport assays used for larvae (*e.g.*, Jaeckle and Manahan, 1989b; Shilling and Manahan, 1994) caused them to cease swimming for as much as 30 s. This might have resulted in underestimates of transport rates. A potential problem with single, end-point rate determinations is that the zero

time point may have a positive y-intercept due to radioactivity retention not associated with transport. In the transport measurements described below, this possible artifact was accounted for by subtracting the radioactivity retained due to nonspecific binding to the animals and filters.

Transport of ^{3}H-labeled alanine and ^{14}C-labeled glucose (2 or 5 μCi per 10 ml; glucose: 353 mCi mmol^{-1}, alanine: 84 Ci mmol^{-1}, New England Nuclear) from seawater was measured by exposing the animals to radiolabeled substrate for a known time (10 to 15 min). The animals were then separated from the radioactive medium using vacuum filtration onto a Nuclepore membrane filter (5 μm pore-size). The animals were washed three times with 5 to 10 ml (each wash) of seawater and were vacuumed to dryness on the filter between each wash. As controls, filters without animals and filters with heat-killed animals (65°C for 10 min) were exposed to radiolabel and washed in an identical fashion. The amount of radiolabel retained by the filter alone, or the filter and dead animals, was always less than 10% of that retained by the filter and live animals, and was subtracted from the total amount of radioactivity on the filter with live animals. The animals on the filter were counted using a dissecting microscope and placed in tissue-solubilizer (Scintigest, Fisher Scientific) for 24 h, after which scintillation cocktail (Bio-HP, Fisher Scientific) was added for liquid scintillation counting. After appropriate quench correction and calculations to determine the moles of substrate transported per individual, affinity (K_t) and maximal transport capacity (J_{max}) were calculated for alanine and glucose using Eadie-Hofstee transformations of transport rate.

Results

Rates of oxygen consumption before and during metamorphosis

Metabolic rates (as oxygen consumption) were determined continuously using coulometric respirometry over periods of days for veligers and plantigrades from three cultures (Fig. 1 and Table I). Swimming veligers (stage ii) had mean metabolic rates (±95% CI) ranging from 197 (±34) to 347 (±40) pmol O_2 individual^{-1} h^{-1} for 1 or 2 days prior to settlement (Cultures E and G, respectively; Table I). The mean metabolic rate for stage ii larvae was 292 (±9) pmol O_2 individual^{-1} h^{-1} for Cultures C, E, and G. Variation among metabolic rates for animals from different cultures may in part be due to the difference in culturing temperature (see Methods) and also batch-to-batch variation (Fig. 3, Jaeckle and Manahan, 1989a). For all three cultures there was a significant increase in metabolic rate coincident with the time when metamorphosis into the juvenile was occurring (*e.g.*, Culture C: stage iii, 222 ± 10 pmol O_2 individual^{-1} h^{-1} to stage iv, 1659 ± 59 pmol O_2 individual^{-1} h^{-1}, Table I; Fig. 1). During this period there was no observable increase in crawling activity, as seen under dissecting and compound microscopes. Plantigrades at stage iv (3 to 4 days post-settlement) were undergoing morphological changes associated with metamorphosis (see Methods section for description of stages).

Metabolic rates of veliger larvae (stage ii, 7-day-old) from Culture G were also determined (in addition to coulometric measurements) by measuring depletion of oxygen from seawater in six sealed 300-ml BOD bottles. The rates measured ranged from 173 pmol oxygen larva^{-1} h^{-1} to 261 pmol oxygen larva^{-1} h^{-1}, with a mean rate of 213 (±35, 95% CI) pmol larva^{-1} h^{-1} for the six independent measurements. This rate measured at 15°C is less than that for sibling stage ii larvae measured using the coulometric respirometry method (Table I, Culture G, 288 ± 34 pmol oxygen larva^{-1} h^{-1}). Both methods gave significantly higher respiration rates than previously reported values obtained using polarographic oxygen sensors (see Discussion).

Changes in biomass before and during metamorphosis

Changes in biomass for five cultures of abalone are shown in Figure 2; an asterisk above a bar represents a significant ($P < 0.05$) difference between the biomass on the stage so marked and that of the day or stage before. In two of the four cultures for which there was an oocyte measurement (Cultures A and D), there was no significant difference (ANOVA: $P > 0.10$) between the biomass of competent veligers (stage ii) and that of the oocytes (stage 0). In both of the other two cultures (Cultures B and C) the veligers had a lesser biomass than the oocytes (Newman-Keuls: $P < 0.05$); for example, in Culture C the veligers (stage iib) lost 17% (0.24 μg) of their biomass relative to the oocytes. During early post-settlement (stage iii, first 2 days), newly settled plantigrades in four of five cultures showed an increase in biomass at some point before metamorphosis had commenced (Fig. 2, Newman-Keuls: $P < 0.05$). These changes occurred either between stages ii and iii or between 2 consecutive days at stage iii (labeled in Fig. 2 as iiia and iiib, respectively). During this time most of the animals still had their vela and were crawling, and by the second day post-settlement the adult shell was beginning to develop. No feeding activity was observed during this time. Of the four cultures (B, C, D, and G), in which the plantigrades (to stage iv) were cultured through metamorphosis, only one (culture B) showed a statistically significant (Newman-Keuls: $P < 0.05$) decrease in biomass from stage iii (1.63 ± 0.049 μg) to stage iv (1.37 ± 0.038 μg). In all three of the cultures (B, C, and D) for which data were

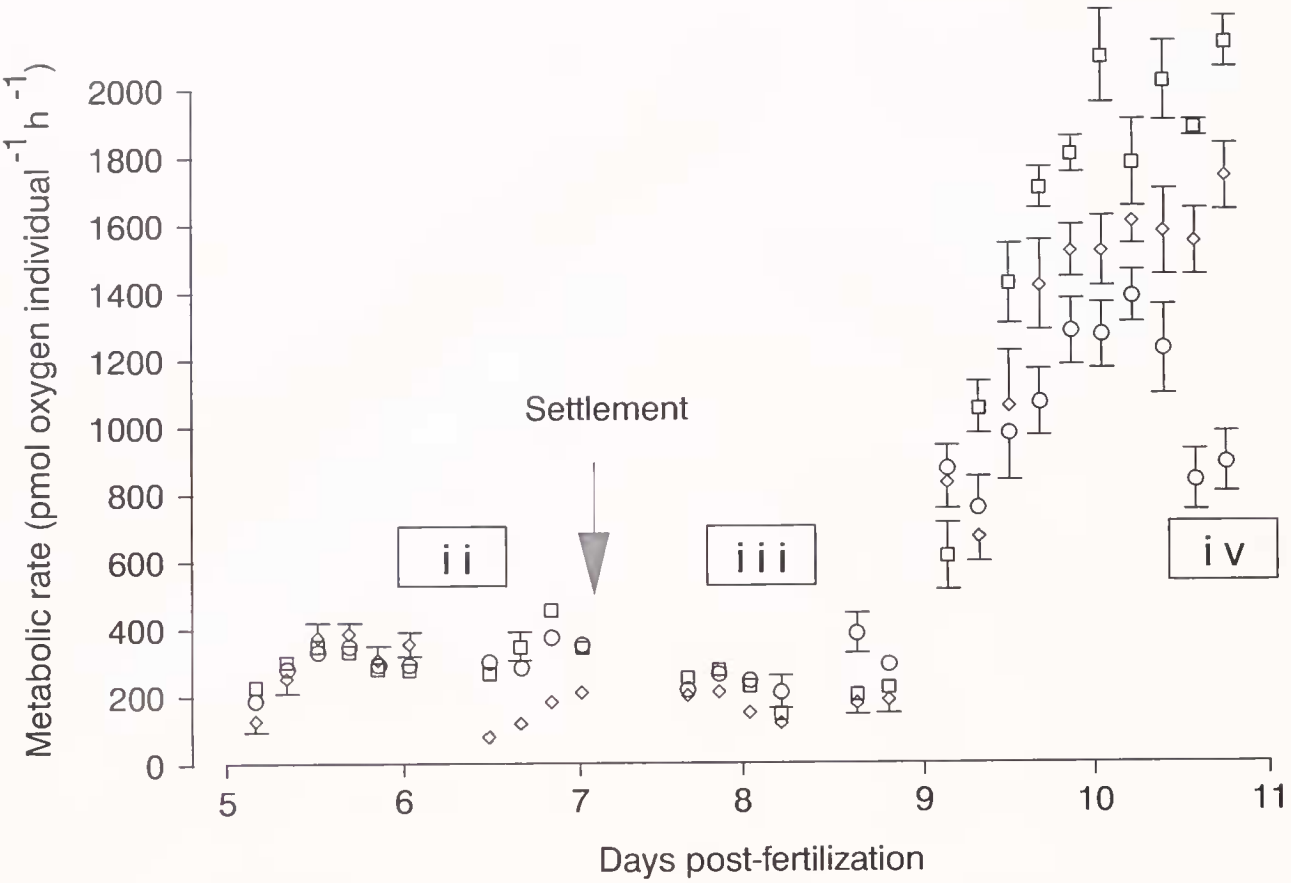

Figure 1. Rates of oxygen consumption of larvae and plantigrades from Culture C. Each symbol represents the mean of twelve 20-min rate measurements, for a total of 4 h. Error bars represent 1 standard error of the mean. Different symbol types represent separate respiration chambers. The gaps between runs represent times when the animals were being replaced with new animals and the chambers cleaned. "Settlement" indicates the time when larvae were treated with γ-amino butyric acid at the AbLab (see Methods). The boxed roman numerals indicate the stage of development (see Methods for definitions).

available to compare oocytes and plantigrades (stage 0 to iv, respectively), there were no significant differences between the oocytes' biomasses and those of the plantigrades (ANOVA: $P > 0.10$).

Meeting metabolic costs with endogenous reserves

Can newly settled plantigrades meet their metabolic needs through catabolism of endogenous tissue reserves? In Table II the energy required to meet metabolic needs was compared to the energy available from changes in aerobically catabolized biomass. The two most important energy reserves for abalone larvae are protein and lipid, total carbohydrate content being below the level of detection (Jaeckle and Manahan, 1989a). Because we do not know how protein and lipid fluctuate during metamorphosis, the value (31.5 kJ g^{-1}) used in Table II to convert biomass to energy was the mean of the values for protein (24.0 kJ g^{-1}) and lipid (39.5 kJ g^{-1}; Gnaiger, 1983). Similarly, the enthalpy of combustion value used for oxygen (484 kJ (mol oxygen)$^{-1}$) was the mean of the values for lipid combustion (441 kJ (mol oxygen)$^{-1}$) and protein combustion (527 kJ (mol oxygen)$^{-1}$; Gnaiger, 1983). Culture C was chosen for these calculations because it had the most complete data set for each developmental stage's metabolic rate and biomass. Developing stages from Culture C (Table II) had 6.35 mJ of available energy for the transition from stage ii (day 6) to stage iv (day 9). The required energy for the same period, calculated from metabolic rates and gains in biomass, was 16.41 mJ. Thus the settled plantigrades could account for only 38.7% of their energy requirements using endogenous reserves.

The biomasses of the oocytes (from Cultures A, B, C, and D) ranged from 1.24 to 1.44 μg with a mean of 1.36 (±0.038) μg (Fig. 2). The hatching of the bars for Culture C (Fig. 2) represents the expected loss of energy as biomass that would be needed to meet the measured metabolic

Table I

Rates of oxygen consumption of larvae and plantigrades before and during metamorphosis

	Days post-fertilization							
	6	7	8	9	10	11	12	13
Culture C (15°C): [stage]	[ii]	[ii]	[iii]	[iv]	[iv]			
Metabolic rate (pmol oxygen individual^{-1} h^{-1})	307 (12)	302 (18)	222 (10)	705 (69)	1659 (59)			
Total oxygen (oocyte to juvenile)	= 113.52 nmol							
Equivalent energy	= 54.95 mJ							
Equivalent biomass	= 1.73 μg							
Culture E (13°C): [stage]				[ii]	[iii]	[iii]	[iv]	[iv]
Metabolic rate (pmol oxygen individual^{-1} h^{-1})				197 (34)	139 (38)	259 (101)	642 (97)	773 (93)
Total oxygen (oocyte to juvenile)	= 86.07 nmol							
Equivalent energy	= 41.66 mJ							
Equivalent biolass	= 1.31 μg							
Culture G (15°C): [stage]	[ii]	[ii]	[iii]		[iv]	[iv]		
Metabolic rate (pmol oxygen individual^{-1} h^{-1})	347 (40)	288 (34)	251 (24)		425 (73)	911 (184)		
Total oxygen (oocyte to juvenile)	= 103.07 nmol							
Equivalent energy	= 49.89 mJ							
Equivalent biomass	= 1.57 μg							

Each value is the mean hourly rate for a given day, with at least 80 measurements (at 20-min intervals, 2 or 3 chambers) used to determine a given mean. The values in parentheses are the 95% confidence intervals of each mean. The "total oxygen" values were calculated by multiplying each hourly rate by 24 h, then adding the daily rates for all of the days from oocyte [stage 0] to early juvenile [stage iv]. The rates for stages prior to the competent veligers (stage ii) were assumed to be the same as the stage ii veliger (see Jaeckle and Manahan, 1989a) and were included in the total oxygen calculation by multiplying by the age (in days). The total oxygen was multiplied by the mean oxyenthalpic equivalent of lipid and protein (484 kJ (mol oxygen)$^{-1}$) to obtain the "equivalent energy." This number was in turn divided by the enthalpic equivalent for aerobically catabolized biomass [(average values for protein and lipid (31.75 kJ g^{-1})] to obtain the "equivalent biomass" needed to meet the metabolic cost. All enthalpic equivalents are from Gnaiger (1983).

needs (from Table I). This decrease in biomass was calculated by converting the measured rate of oxygen consumption for a given day (Table I) into a biomass-equivalent (calculated using values from Gnaiger, 1983). This biomass value was in turn subtracted from the calculated biomass of that day to give the expected biomass for the subsequent day. The equivalent biomass that would be needed to meet the calculated metabolic costs for the entire development, from oocyte to juvenile, ranged from 1.31 to 1.73 μg (Cultures E and C, respectively, Table I). These results are striking as they mean that *all* of the initial energy reserves (biomass) in the oocyte are needed for development, but none are used, as is evident from a comparison of oocyte and juvenile biomasses.

Rates of alanine and glucose transport during metamorphosis

Plantigrades (at 20°C, Culture D) 3 days after settlement (stage iv) had a 3-fold higher maximal transport capacity for alanine (J_{max}) = 182.0 ± 49.2 [SE] pmol individual^{-1} h^{-1}, K_t = 96 μM) than that of the stage ii veligers (J_{max} = 61.2 ± 1.9 pmol individual^{-1} h^{-1}, K_t = 29 μM) (Fig. 3A, B). In contrast to amino acid transport, the capacity for glucose transport decreased from stage ii (28.7 ± 5.1 pmol individual^{-1} h^{-1}, K_t = 19 μM) to stage iv (14.5 ± 3.0 pmol individual^{-1} h^{-1}, K_t = 27 μM) (Fig. 3C, D). A similar full kinetic analysis showed an increase for plantigrades of Culture F (at 12°C) where the J_{max} for alanine increased 2-fold from 27.9 ± 16.7 pmol individual^{-1} h^{-1} for stage iii to 51.5 ± 6.1 pmol individual^{-1} h^{-1} for stage iv (data not shown as a figure). These increases were concomitant with both the morphological changes and increase in metabolic rates associated with metamorphosis.

Discussion

Maternally endowed reserves in the egg, or reserves accumulated during the development of subsequent feeding larval forms, have been considered to be the sole source of energy for marine invertebrates during nonfeeding stages of development (*e.g.*, metamorphosis of the barnacle: Lucas *et al.*, 1979; the bivalve: Holland and Spencer, 1973; Rodriguez *et al.*, 1990). However, studies with the bivalve *Crassostrea virginica* showed that stages undergoing

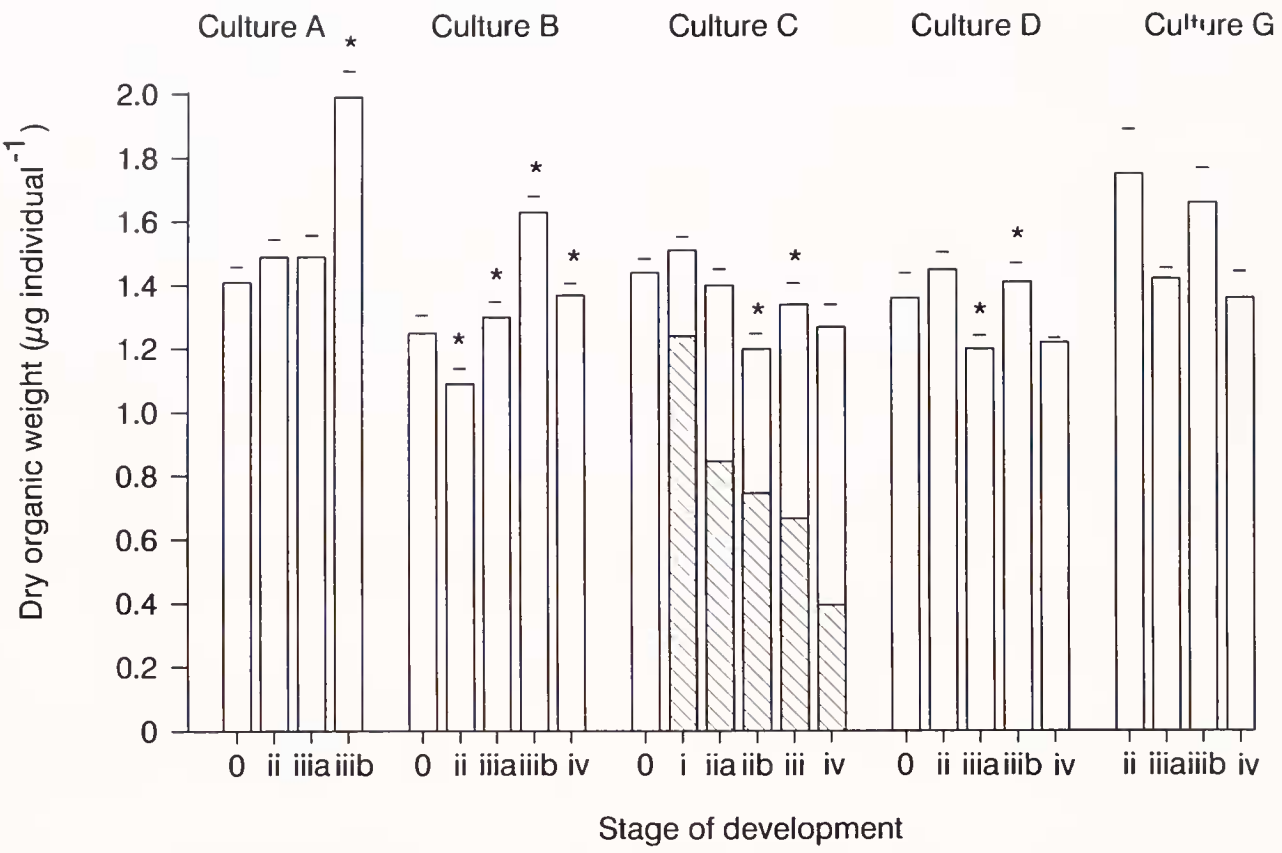

Figure 2. Biomass changes for cultures A, B, C, D, and G, from oocyte or veliger to plantigrade. Stages of development (0 to iv) are indicated on the *x*-axis and are described in the Methods section. Use of 'a' and 'b' after a stage represents a second sample (on another day) taken of the same stage. The line above each bar represents 1 standard error of the mean. The values for bars indicated by the asterisks (*) are significantly different ($P < 0.05$) from the values for bars of the stage immediately prior to them. For example, for Culture B stage ii is significantly lower than stage iiia. The hatching of the bars for Culture C indicates the expected decrease in biomass due to aerobic catabolism of endogenous organic reserves (rates of oxygen consumption values from Table I, conversion factors from Gnaiger, 1983).

metamorphosis can feed on phytoplankton (Baker and Mann, 1994). "Feeding" during bivalve metamorphosis can also occur *via* uptake of dissolved organic material (DOM) (Manahan and Crisp, 1983). Previous studies (Jaeckle and Manahan, 1989a) with the lecithotrophic larvae of *Haliotis rufescens* showed that depletion of endogenous reserves did not account for the energy requirements during larval development. In the present study, we confirm that finding and extend the conclusion of energy imbalance to stages undergoing metamorphosis from larva to juvenile. Only a small fraction of the total cost of larval development and metamorphosis could be accounted for by depletion of endogenous reserves (as measured by changes in dry organic mass, Fig. 2). There was no statistically significant loss of biomass from the oocyte to the juvenile stage for the cultures studied, even though at least 75% of the oocytes' biomass should have been used to account for metabolic cost of development to the juvenile stage (Fig. 2, Culture C shaded areas).

Metabolic costs of metamorphosis

There was an increased metabolic cost associated with metamorphosis of *H. rufescens* (Table I). At about 2 days after induction of settlement, the plantigrade stages had an increased metabolic rate (day 9) compared to earlier stages of swimming veligers and newly settled plantigrades. This increase in metabolism continued until the measurements were discontinued at day 11 (Fig. 1, Culture C). There was no corresponding increase in biomass for stages of Culture C during this period of development (Fig. 2). One possible explanation for the increase in metabolic requirements is that it is energetically expensive to rearrange existing tissue and construct new ones, and that the observed increase in metabolic rate may reflect the energy cost of metamorphosis to the juvenile stage. Regardless of mechanism, increases in metabolic rates have been measured in other species undergoing tissue rearrangement (sea urchin embryos, Immers and Runn-

Table II

The contribution of endogenous reserves (biomass) to metabolic demands during metamorphosis for Culture C

		Days post-fertilization		
		6–7	7–8	8–9
Changes in biomass:				
biomass (μg)		−0.20	+0.41	0
equivalent energy (mJ) (31.75 kJ g^{-1})		−6.35	+4.45	0
Sum of available energy (mJ) (from decrease in biomass)	= −6.35			
Metabolic demand:				
metabolic rate (pmol d^{-1})		7308	6288	11,124
equivalent energy for days 7 to 9 (72-h period) (mJ) (484 kJ mol^{-1})		+3.54	+3.04	+5.38
Sum of required energy (mJ)	= +16.41			
gain in biomass = +4.45				
plus metabolic rate = +11.96				
Energy balance: Ratio of available to required energy	38.7			

Biomass values are from Figure 2; metabolic rates are calculated from those in Table I. The rates given per day are the average hourly means for the days encompassed by the period (*e.g.*, "6–7" refers to days 6 and 7), multiplied by 24 h; for example, the metabolic rate in the period "6–7" is the average of the rates on days 6 and 7. Enthalpies of combustion used to convert changes in biomass to "equivalent energy" and the oxyenthalpic equivalent are from Gnaiger (1983, as used in Table I). "+" refers to gains in biomass or required energy for metabolism; "−" refers to losses in biomass. Stages of development corresponding to age in days are given in Table I and Figure 1.

strom, 1960; metamorphosing barnacles, Lucas *et al.*, 1979).

The values for rates of oxygen consumption presented here for larvae of *H. rufescens* are higher than previously reported (Jaeckle and Manahan, 1989a). In that study the rate of oxygen consumption measured with a polarographic oxygen sensor was 84 pmol oxygen $larva^{-1}$ h^{-1}, compared to the present values of 197 to 347 pmol oxygen $larva^{-1}$ h^{-1}, measured with coulometric respirometry, and 213 pmol oxygen $larva^{-1}$ h^{-1}, measured with Winkler titration. Concerns about the accuracy of polarographic oxygen sensors for measurements of larval metabolic rates are discussed in detail elsewhere (Hoegh-Guldberg and Manahan, 1995). Note, however, that the higher metabolic rates now measured for larvae of *H. rufescens* do not negate the earlier conclusion of Jaeckle and Manahan (1989a) that endogenous reserves do not fuel metabolic demands and that an exogenous energy source (*i.e.*, DOM in seawater) contributes to metabolic costs. Given higher values for metabolic rates, the calculated contribution from endogenous reserves would be even lower than estimated by Jaeckle and Manahan (1989a). Nor does the use of the lower value for metabolic rate (84 pmol oxygen $larva^{-1}$ h^{-1}) alter the conclusion of the present study that rates of biomass loss cannot account for metabolism through metamorphosis. This is based on the following calculation: the cumulative oxygen consumption over a 10-day period (to stage iv) would be 19.2 nmol O_2 (at 84 pmol oxygen $larva^{-1}$ h^{-1}), equivalent to 0.30 μg of biomass (conversion of oxygen to biomass: Gnaiger, 1983). With a starting mean oocyte biomass of 1.36 μg (Fig. 2, four cultures), the theoretical mass of a stage iv plantigrade would be 1.06 μg, given a biomass loss to fuel metabolism of 0.30 μg. A value of 1.06 μg is still less than any of the biomasses measured for plantigrades from all cultures.

Meeting the increased metabolic costs: roles of DOM and endogenous reserves

DOM in seawater may contribute significantly to the energy and growth requirements of marine invertebrate embryos and larvae (abalone: Jaeckle and Manahan, 1989a; sea urchins: Shilling and Manahan, 1990; Shilling and Bosch, 1994). In the present study, newly settled larvae (plantigrades), maintained in continuously flowing seawater, gained or did not change in biomass prior to their being able to feed on particles. This is in contrast to other studies with newly settled molluscs, raised in batch cultures, where loss of endogenous reserves has been observed (*e.g.*, Rodriguez *et al.*, 1990). Our results indicate that both veligers and settled plantigrades of *H. rufescens* may meet most of their energy requirements through the uptake and metabolism of an exogenous food source, such as DOM. We calculated that plantigrades used their endogenous reserves to fuel only 39% of the metabolic cost of metamorphosis (days 6–9, Culture C, Table II). An exogenous source of energy has to be used to meet the remaining 61% of the energy costs. Upon metamorphosis, the maximum capacity for alanine transport (J_{max}) increased 3-fold (Fig. 3A, B) from stage ii to iv, with the sites of uptake being the velum (Dimster-Denk

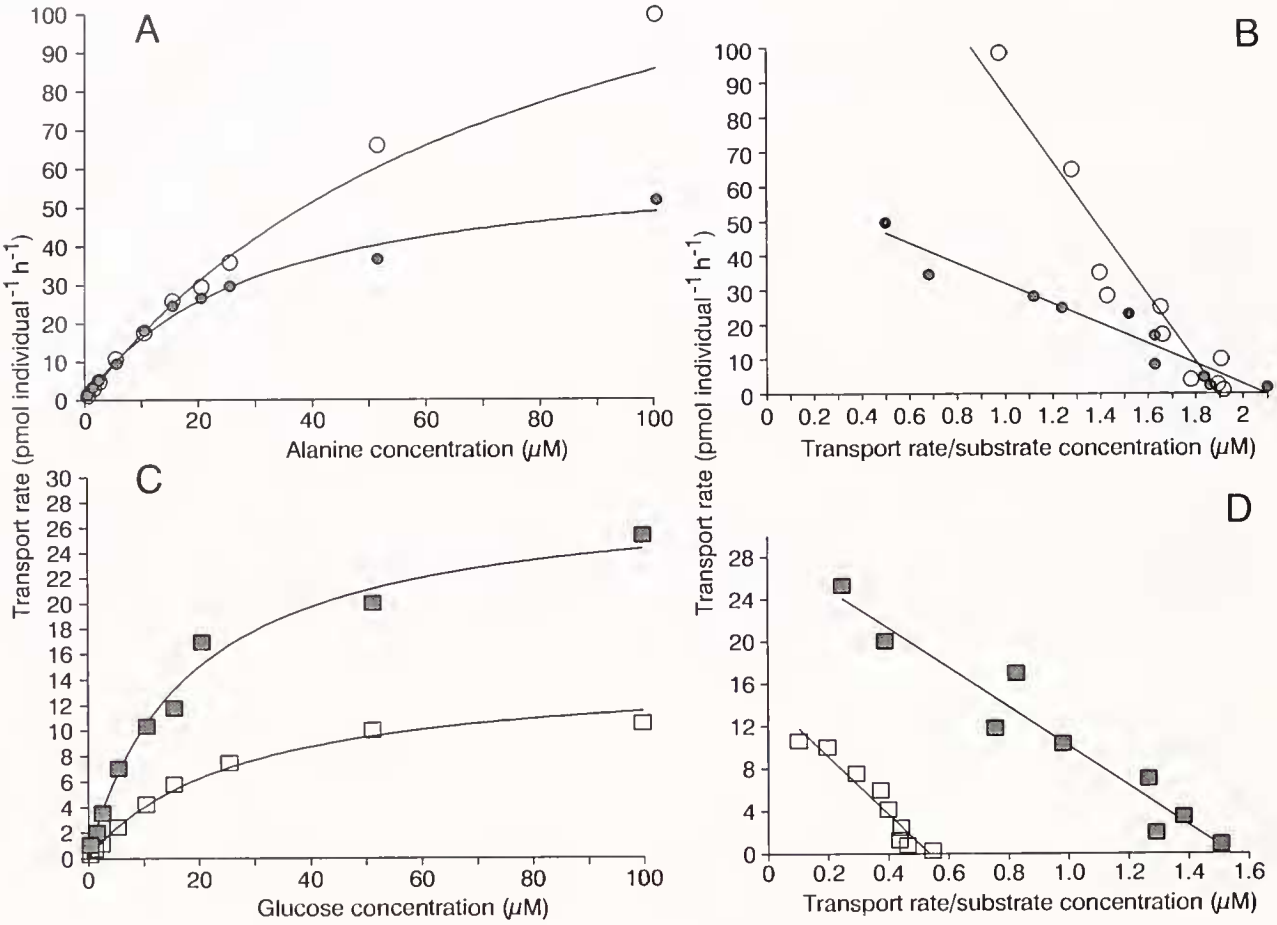

Figure 3. Kinetics of alanine (A & B) and glucose (C & D) transport by competent veligers (stage ii, closed symbols) and settled plantigrades (stage iv, open symbols). Each symbol represents a single uptake measurement; the curves were calculated using the K_t and J_{max} for each stage. The K_t and J_{max} values were as follows: (stage ii) alanine, 29 μM, 61.2 pmol alanine larva^{-1} h^{-1}; glucose, 19 μM, 28.7 pmol glucose larva^{-1} h^{-1}; (stage iv) alanine, 96 μM, 182.0 pmol alanine larva^{-1} h^{-1}; glucose, 27 μM, 14.5 pmol glucose larva^{-1} h^{-1}. (B) and (D) are Eadie-Hofstee plots of the data presented in (A) and (C), respectively.

and Manahan, unpub.) and presumably the developing gill buds in *H. rufescens* (*cf.* bivalve metamorphosis: Manahan and Crisp, 1983). The change in alanine transport kinetics is probably due to an increase in the number of transporter sites (indicated by increase in J_{max}) and a transition from localization of transport in the velum (once it is lost) to transport by other organs, such as the developing gill. Not all transport systems increase in capacity; the J_{max} for glucose transport decreased by half during metamorphosis of a swimming larva (stage ii) to a plantigrade (stage iv). However, if glucose transporters are present in the gill tissue, as is the case for adult mussels (Wright, 1988), then maximum transport capacity for sugars may increase as this tissue develops further.

The total oxygen consumed from oocyte to juvenile stage in 10 days of development was equivalent to 1.73 μg of biomass, more than the starting material in the oocyte (Culture C: Table I, Fig. 2). Could the transport of DOM from seawater supply an amount of energy to lecithotrophic development equal to the initial maternal investment of energy in the oocyte? An estimate of the input of DOM can be obtained as follows. Based on maximum transport capacities (Fig. 3), glucose would be transported at a rate of 28 pmol h^{-1} for a larva, decreasing to 14 pmol h^{-1} for a juvenile. Over the 10-day period of development for which we have measurements of metabolic rates, these transport rates would provide 1.1 μg of organic material as glucose (8-day larval period at a rate of 28 pmol larva^{-1} h^{-1} = 968 ng glucose; 2-day juvenile period at a rate of 14 pmol larva^{-1} h^{-1} = 121 ng glucose). Similarly, during this 10-day period, alanine transport would yield 1.8 μg of material (8-day larval period at a transport rate of 61 pmol larva^{-1} h^{-1} = 1042 ng alanine; 2-day juvenile period at a transport rate of

182 pmol juvenile^{-1} h^{-1} = 777 ng alanine). Thus at maximum transport capacities, the combined input of alanine and glucose over the 10 days of development would be 2.9 μg (1.8 μg alanine plus 1.1 μg glucose). This is more material than is required to supply the 1.73 μg of biomass calculated to meet metabolic costs (Culture C, Table 1). Although unknown at this time, the substrate concentrations of dissolved amino acids and sugars in the pelagic (larva) and benthic (juvenile) environments of these animals are unlikely to be high enough for the transporters to reach J_{max} (concentrations of *ca.* 100 μ*M* would be needed, see Fig. 3B). Nonetheless, even if the amino acid and glucose transporters were to operate through development at only 20% of their maximum capacity, this would result in transport from the environment of about one-third of the 1.73 μg of organic material required to supply metabolism (2.9 μg × 0.2 = 0.58 μg; 0.58/1.73 = 0.33). For transport rates to reach 20% of J_{max} in veliger larvae, amino acids would have to be at a concentration of 7 μ*M* and sugars at 5 μ*M*—the high end of reported concentrations, even in near-sediment waters (Williams, 1975). The metabolic requirement for high substrate concentrations in larvae of *H. rufescens* is largely set by the measured high K_t value of 29 μ*M*. This value is consistent with previous studies in which a K_t value of 23 μ*M* for alanine transport was reported for 2-day-old veligers of *H. rufescens* (Manahan *et al.*, 1989). Relative to the K_t values for amino acid transport reported for other species of marine invertebrate larvae (Manahan, 1990), veligers of *H. rufescens* have lower-affinity transporters (*i.e.*, higher K_t values). It is noteworthy that if abalone veligers had K_t values in the low micromolar range (similar to those reported for sea urchin larvae; Manahan *et al.*, 1989), an amino acid concentration of only 0.25 μ*M* would be required to have a transport rate of 20% of J_{max} in *H. rufescens*. Further study is required to understand the physiological significance of the range of K_t values found in different larval species and at different stages of development. Also, dissolved free amino acids and monomeric sugars in seawater make up only a small proprotion of the total pool of DOM (Williams, 1975). The contribution that other components of the DOM pool might make to these animals is unknown, but their transport from seawater would only increase the total contribution of DOM to the energetics of development.

Metabolic rates increased 3- to 5-fold during metamorphosis (Table 1), while J_{max} for alanine increased 3-fold (Fig. 3). This phenomenon of up-regulation of maximum transport capacity for amino acids as metabolic demand increases during metamorphosis is consistent with other studies of animal development (mammals: Ferraris and Diamond, 1989; marine invertebrates: Manahan *et al.*, 1989). As the settled larva metamorphoses it must supply the increased metabolic costs. An increased capacity to transport dissolved organic nutrients would provide a mechanism for meeting the higher costs of metamorphosis. Larvae undergoing metamorphosis have sufficient energy reserves to supply these metabolic needs but, if available, exogenous sources will be used in preference—resulting in individuals of greater biomass that are better able to survive further stresses (*e.g.*, delayed feeding) and potentially have higher survival rates as juveniles.

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